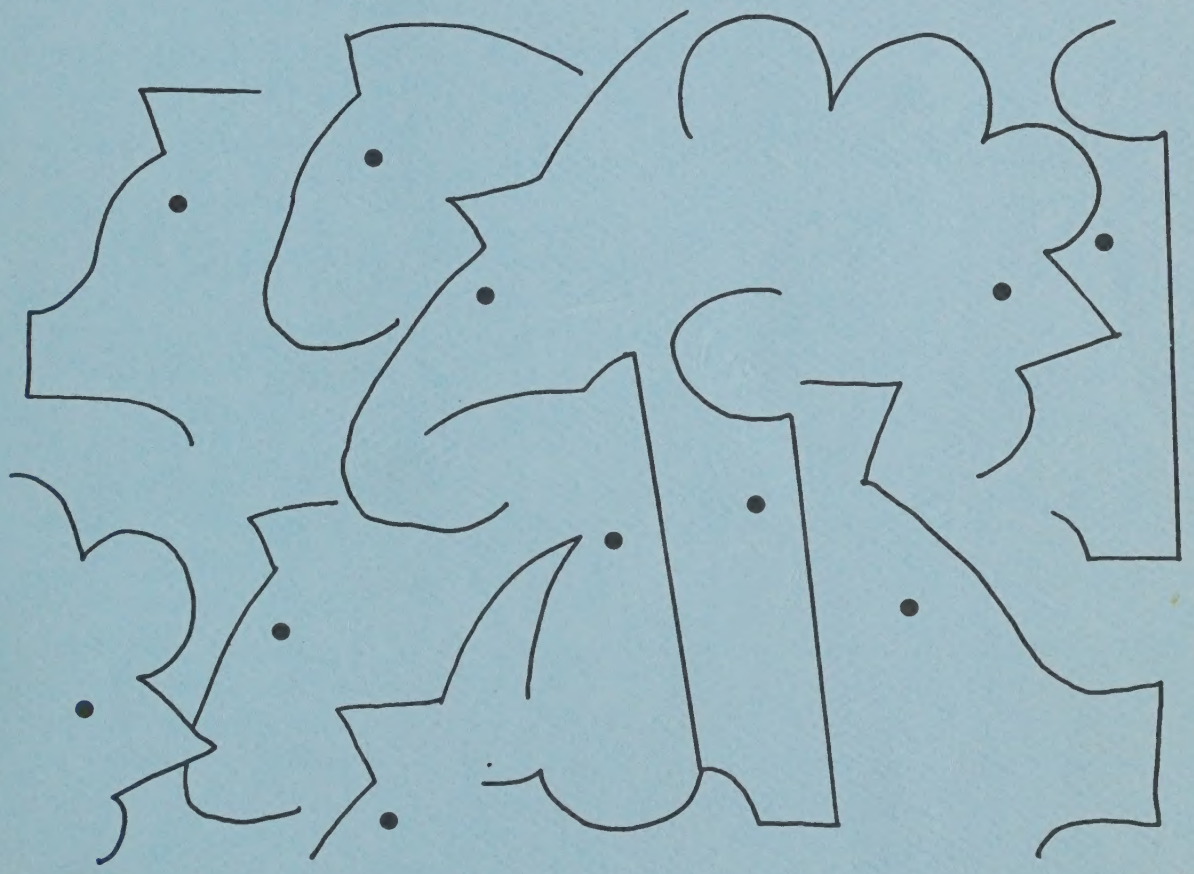
Diagnosis of African Swine Fever

(Hemadsorption Test)

PLUM ISLAND ANIMAL DISEASE CENTER



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SCIENCE AND EDUCATION ADMINISTRATION

FEDERAL RESEARCH
NORTHEASTERN REGION

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DIAGNOSIS OF AFRICAN SWINE FEVER (ASF) BY THE HEMADSORPTION TEST

African swine fever resembles hog cholera (HC) closely in regard to clinical and pathological aspects. However, it is a separate disease entitity caused by a virus which is neither morphologically nor immunologically related to the causative agent of HC. The virus of ASF has been found in three species of wild swine indigenous to Africa. They serve as a reservoir of the virus without manifesting any clinical signs of illness. When ASF virus is transmitted to domestic swine, however, it usually produces a highly contagious, peracute, febrile and septicemic disease chracterized by marked hemorrhages in the internal organs and cyanosis of the skin. Mortality often approaches 100%. However, in areas where the disease has become enzootic in domestic swine, the mortality is lower. Subacute and chronic infections are common and the resemblance to HC is more pronounced (Hess, W. R., 1971 African Swine Fever, Virology Monographs 9, 1-33 by Springer-Verlag).

Since it is usually difficult to differentiate ASF from HC (and even some other diseases) by such methods as observation of clinical signs and pathology, the final requirement for diagnosis is a laboratory test. Several such tests are in use, including complement fixation (CF), fluorescent antibody (FA), agar diffusion (AD), immuno-electroosmophoresis (IEOP), and hemadsorption (HAd). The last test is especially valuable in diagnosis of the acute disease and is effective in testing specimens taken just before the rise in temperature to the stage immediately prior to antibody formation in the host animal. The HAd test will be described in this microfiche.

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THE HEMADSORPTION (HAd) TEST

The HAd test is a simple one, composed of the following basic components and procedures:

1. Leukocyte cultures from the blood of healthy pigs, +
2. Extracts of appropriate tissues, including blood from ASF suspect pigs, +
3. Controls, +
4. 37°C incubation =
5. Hemadsorption in positive cultures.

PROTOCOL FOR HAd TEST

I. Using leukocyte (buffy coat) cultures prepared from normal swine blood.

A. Preparation of cultures

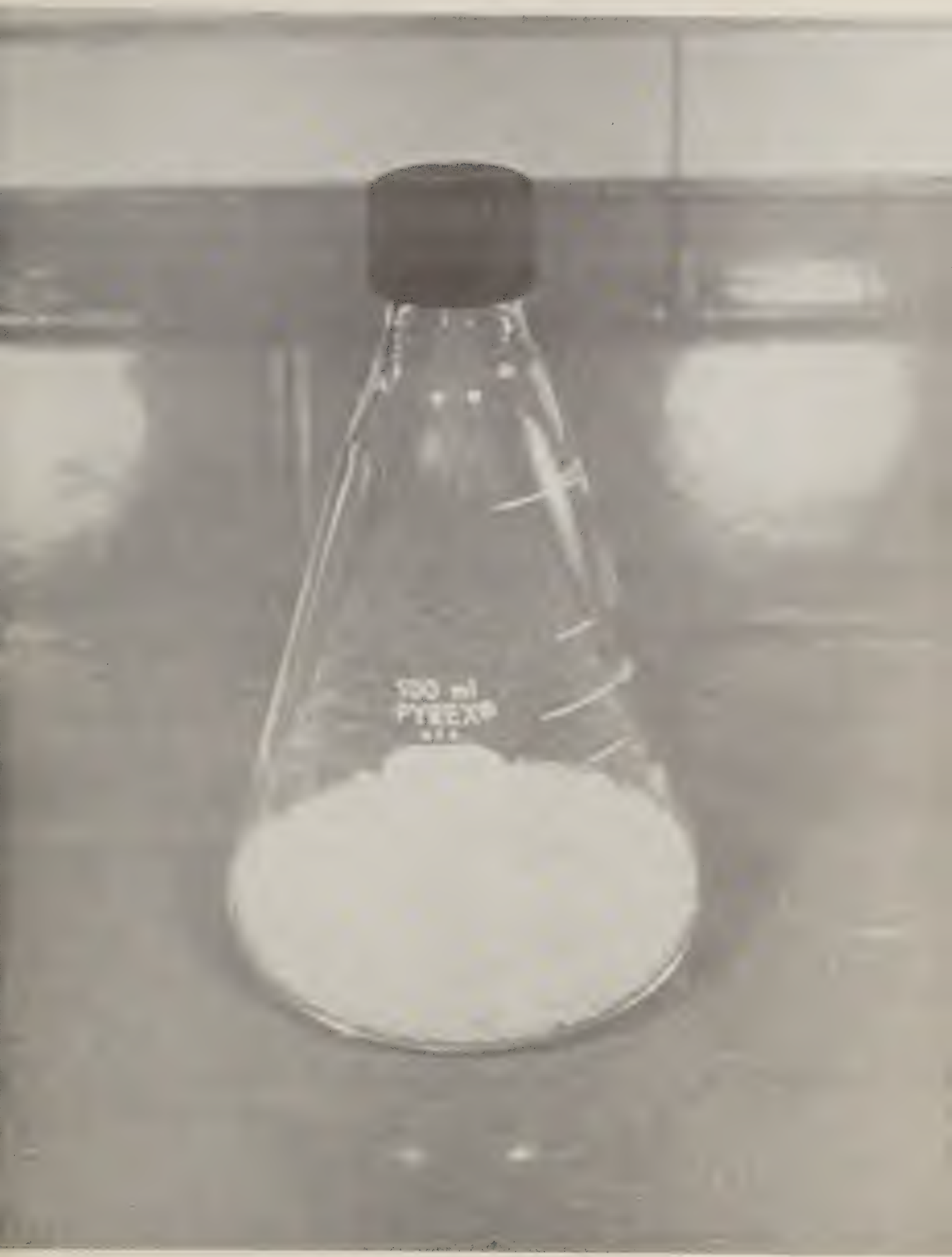
1. Sterile equipment, glassware and supplies should be used throughout.

2. A hood or cubicle is advantageous. All surfaces, including the floor, should be cleaned with antiseptic solution (to keep down aerosols). Air free of particles is desirable.

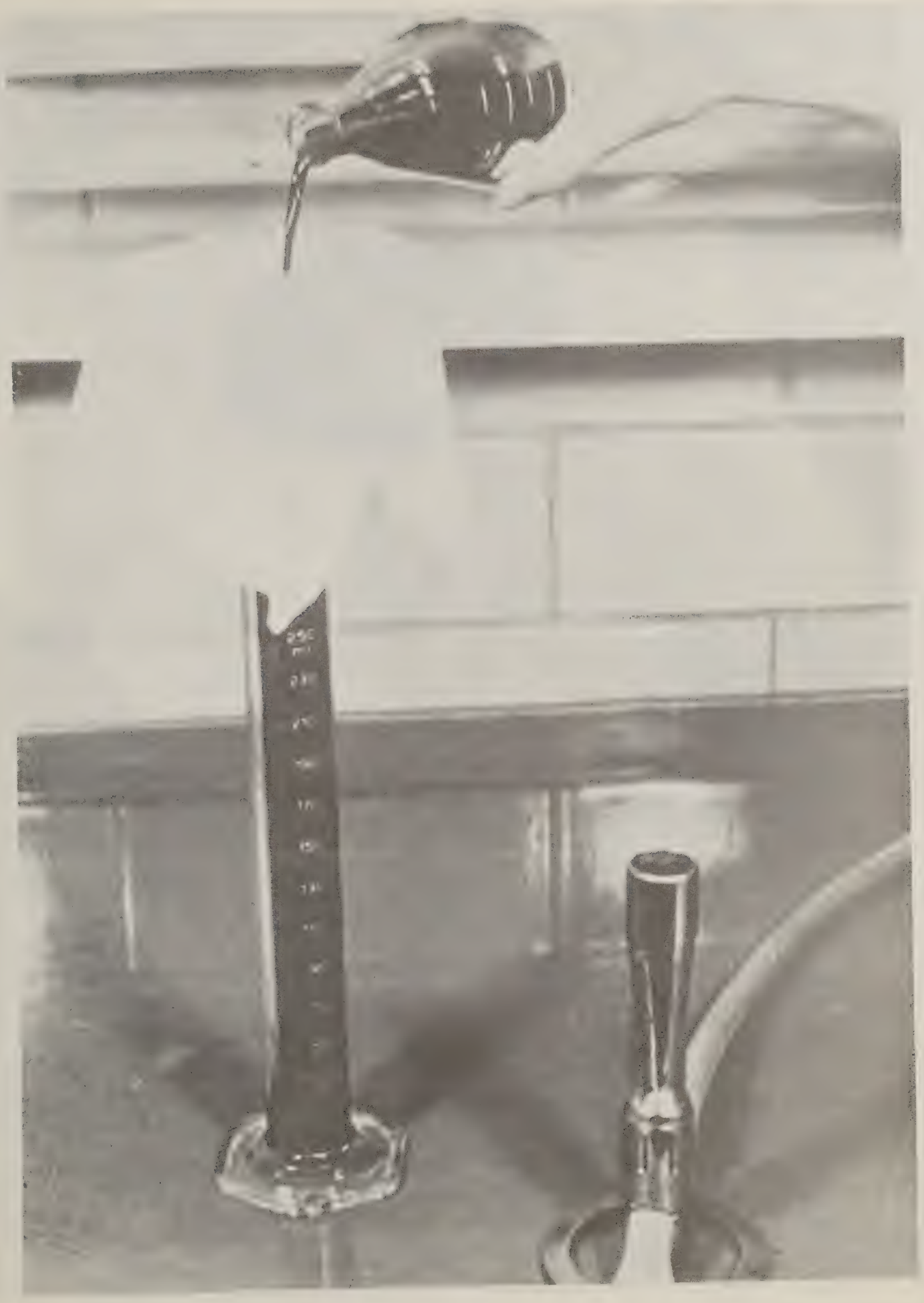
A. 3. Collection and defibrination of blood:

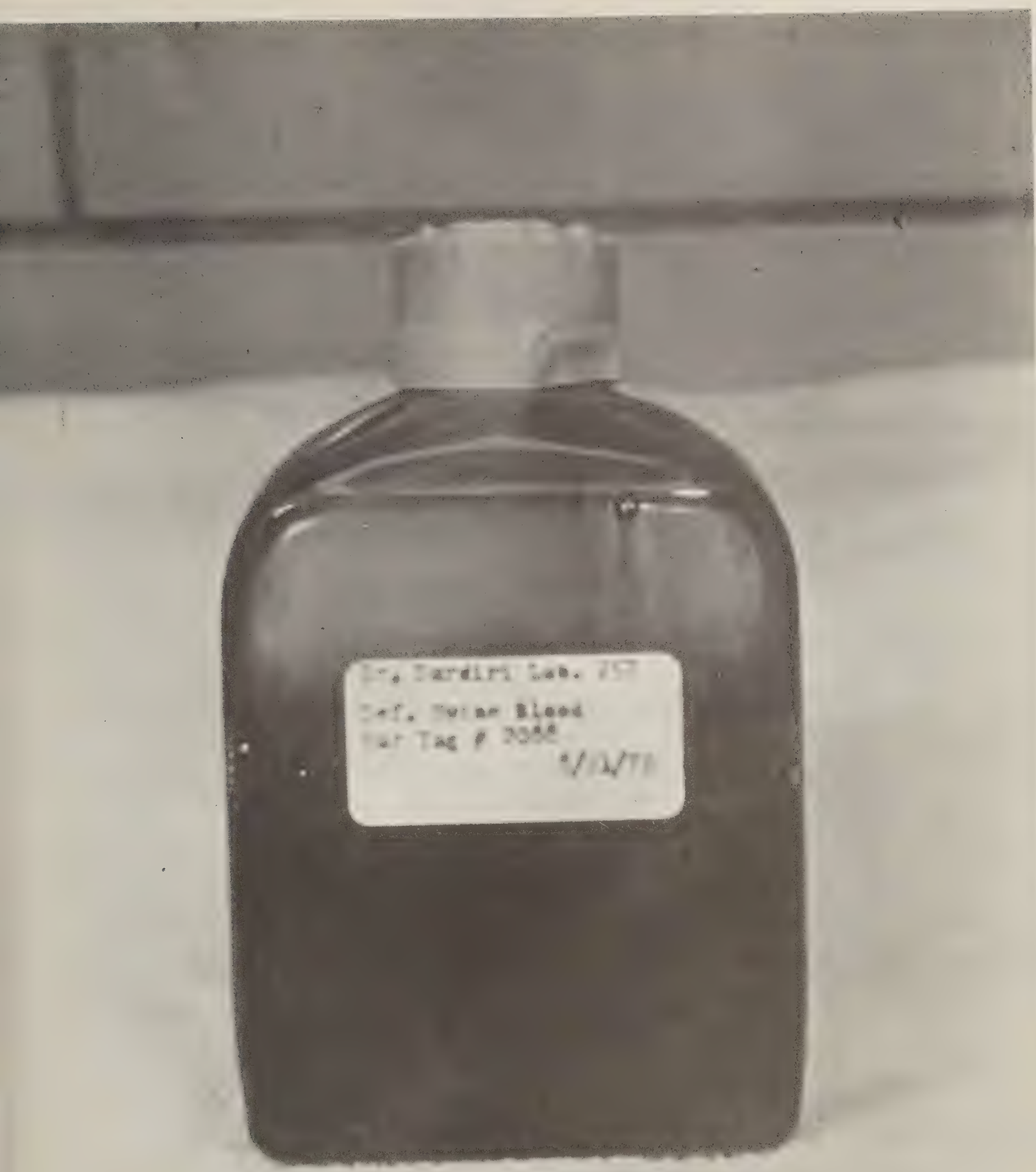
Blood is withdrawn from anterior vena cava into a flask containing glass beads. The flask is shaken as the blood is collected and until defibrination is complete.

The blood is then poured through sterile gauze to remove the beads and clotted fibrin.


100 ml
PYREX®


500
400
300




Def. Tarsier Blood
9/12/71

A. 4. Separation and collection of serum and buffy coat (BC):

a. Antibiotics (Penicillin, 100 units streptomycin, 100 ug per ml.) are added.

b. Centrifugation: The defibrinated blood is centrifuged at 1000 G for 30 minutes in a swinging bucket rotor.

A. 4. c. This centrifugation sediments the erythrocytes and leukocytes in two layers, with the former on the bottom. The leukocytes comprising the BC are in the pink layer below the serum.

A. 4. d. Collection of BC: A syringe and cannula or pipette fitted with a rubber bulb or suction tube may be used to gather the BC layer which lies above the sedimented erythrocytes. A 13 gauge cannula is generally used. The tip of the cannula is allowed to touch, but not dip below, the surface of the packed cells and is moved back and forth across the surface as gentle suction is applied. Often the leukocytes adhere loosely to one another, forming a somewhat contracted mantle that may come off as a single mass without moving the cannula.

A. 4. e. Erythrocytes are required in the cultures, but the quantity drawn off with the BC is usually more than sufficient.

A. 4. f. Removal of the serum:

Some experienced technicians remove the BC prior to removing the serum. Others prefer to remove the serum first. This must be done without disturbing the BC layer or the sedimented erythrocytes. (When the serum is removed first, a small amount, about 3mm in depth, may be left above the BC). The serum is transferred to a flask containing a magnetic stirring bar.

A. 4. g. The BC is washed in the following phosphate buffered saline (PBS) solution:

NaCl	.8 gm
KCl	0.2 gm
$Na_2HPO_4.7\ H_2O$	2.172 gm
KN_2PO_4.	0.2 gm
Dist. H_2O to make.	1000 ml

A. 4. h. (The BC is washed by centrifugation in conical 40 ml or 12 ml centrifuge tubes at 1000 G for 20 minutes.)

30
10
8
2

A. 4. i. Preparation of the leukocyte suspension:

The leukocytes and accompanying erythrocytes are added to the serum and are kept in suspension by magnetic stirring. The number of leukocytes per ml of suspension may be varied over a considerable range and still yield cultures which are satisfactory for virus detection. As a simple rule to follow, 30 to 40 one ml cultures may be prepared from 100 ml of defibrinated blood by the procedures shown.

A. 4. j. There are two criteria to be considered in regulating the number of erythrocytes present in the culture. (1) The number must be sufficient to assure having at least 20 in the immediate vicinity of each leukocyte. Since large numbers of erythrocytes may be phagocytized as the cultures age, the suspensions should contain from 50,000 to half a million erythrocytes per mm^3. A 2% erythrocyte suspension is usually correct. (2) The upper limit to the number of erythrocytes is governed only by the need for adequate illumination when examining the cultures microscopically. Sufficient light is required to penetrate the fluid portion of the culture.

A. 4. k. Dispensing cultures:

The cell suspension is distributed in 1 ml amounts in culture tubes. Leighton tubes are recommended because the flat surface greatly facilitates microscopic examination. If Leighton tubes are not available, 16 X 150mm culture tubes may be used. They must be incubated in a slightly tilted position and should be marked so the culture may always be readily placed in the same position during incubation. Tubes should be stoppered with rubber-lined screw caps, gum or silicone rubber stoppers. The cultures are incubated at 37°C. They may be usable within 24 hours and remain usable up to 5 or 6 days.


234+5 ARS

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B. Inoculation of Cultures

1. Collection of specimens:

Although virus may be isolated from practically any organ and tissue in acute ASF, spleen, the gastro-hepatic lymph node and whole blood are the preferred tissues to sample. They should be collected aseptically from sick or recently dead animals. If they cannot be delivered to the laboratory within a couple of hours, they should be chilled in ice or frozen immediately. When this method is not feasible, blood should have a minimum of 1000 units of penicillin and 1000 ug of streptomycin per ml added; other tissues may be immersed in PBS, physiological saline or any tissue culture medium containing at least 1000 units of penicillin and 1000 ug of streptomycin per ml. The specimens should be transported to the laboratory as quickly as possible.

B. 2. Preparation of inoculum:

In the laboratory, the solid tissues are dispersed by grinding or are finely minced with scissors in a small amount of physiological saline, PBS or any kind of tissue culture fluid. A 20% suspension is usually prepared.

B. 3. Inoculation of BC culture with prepared specimens: Cultures 24 to 48 hours old are most suitable for demonstrating typical HAd within 24 hours. However, cultures may be inoculated the same day they are prepared; in this case, typical HAd may be delayed a few hours before it appears. At least 4 cultures should be inoculated, each receiving 0.2 ml of the tissue suspension (or 0.2 ml of blood). With spleen, excellent results may be obtained by placing 2 or 3 small tissue fragments (about 1 mm^3 in size) directly on the cell layer. (Uninoculated cultures serve as controls.)

C. The Hemadsorption reaction:

1. The normal buffy coat culture:

In the mature buffy coat culture (48 hours), the vast majority of cells seen adhering to the glass may be described as macrophages or monocytes. They are large mononuclear cells with abundant cytoplasm which usually contains some erythrocytes and other phagocytized material. Since these cells are much larger and far more abundant than the macrophages seen in smears prepared from the original cell suspension, it is reasonable to assume that most of them are transformed from the smaller mononuclear cells which were abundant in the original blood.

C. 2. When examining the buffy coat culture, the tube should be rocked gently but sufficiently to dislodge any loosely adhering erythrocytes. The tube is then tipped over to allow the red cells to drain away from the leukocyte cultures. The tube is next placed on the microscope with the side bearing the adhering cells uppermost. It is examined under the 10X objective.

C. 3. The positive HAd reaction:

In an ASF-virus-infected culture prepared for examination as described above, varying numbers of leukocytes will be seen with erythrocytes attached to their surfaces, forming rosette-like patterns. The entire surface of the leukocyte may be covered, presenting a raspberry-like appearance. Large numbers of erythrocytes may be absorbed several layers deep around a single infected leukocyte. The red cells are quite firmly attached and can stand rather vigorous agitation wthout dislodging. In fact, the infected leukocyte may become detached from the glass and may be seen floating across the field with the erythrocyte still attached to its surface. The erythrocyte,also, may gradually become dislodged from around the leukocytes with the culture in the inverted position during prolonged examination.

C. 3. The positive HAd reaction (continued):

Although the number of leukocytes displaying HAd is indicative of the amount of virus present, a single cell displaying typical HAd is sufficient to declare the culture positive. Usually, if such a culture is held another day, the infection will extend and more cells will be involved in the HAd reaction.

C. 4. Non-specific observations:

In cultures more than 48 hours old, macrophages are seen that have phagocytised large numbers of erythrocytes. Such cells may superficially resemble a positive HAd reaction. On close examination, the erythrocytes are clearly seen to be lying within the cytoplasmic membrane of the macrophage.

At other times, for reasons not fully understood, red cells may form clusters or agglutinate. Some of these clusters may become trapped among the leukocytes and somewhat resemble hemadsorption. However, the clusters are usually more irregular in form, are more easily dispersed, and - with a little more agitation - will become dislodged from the leukocytes.

II. HAd in leukocyte cultures prepared from the blood of the infected animal.

A. Introduction:

Since ASF is primarily an infection of the reticuloendothelial system, virus may usually be detected in the blood of the sick animal, especially during the febrile periods. The simple technique described here is not intended as a replacement for the regular HAd test. Firstly, it can only be performed with the blood of a live animal. Secondly, the blood sample must be received in the laboratory a couple of hours after it is drawn. If these conditions can be met, it is sometimes a means of demonstrating HAd within a few hours in acute ASF.

A. Introduction: (continued)

Also, from limited experience with experimentally infected animals, the method appears to be a more sensitive and, certainly, a more convenient means of detecting virus in the subacute and chronically infected animal than is the regular HAd test. The greater sensitivity may be due to the testing or a larger sample of virus.)

B. Method:

1. Ten ml of blood are withdrawn aseptically from the anterior vena cava and transferred immediately to a tube containing 0.3 ml of a 1.0 per cent solution of heparin and quickly mixed.

   In the laboratory, 1000 units of penicillin and 1 mg of streptomycin are added; the blood is distributed to five culture tubes (2 ml per tube). After 5 or 6 hours incubation at 37°C, most of the blood from one tube may be poured off and replaced with 2 ml of Eagle's minimum essential medium containing 10 per cent fetal calf serum. A number of other media serve equally well. The tube is then examined microscopically. In blood from an acutely infected pig, HAd may already be discernible. If a roller drum is available, the blood may be dispensed in 16 X 150 culture tubes and rolled at 12 revolutions per hour.

B. 2. This method facilitates the attachment of leukocytes to the glass and apparently provides better aeration for cultures that are to be maintained for several days when attempting to detect virus in blood from chronically infected pigs. In any case, a culture is removed daily, and the blood is replaced with tissue culture medium and examined for HAd. If negative, the culture is returned to the incubator and examined daily thereafter for at least seven days.
If HAd occurs, it has the same appearance as that seen in the regular test.